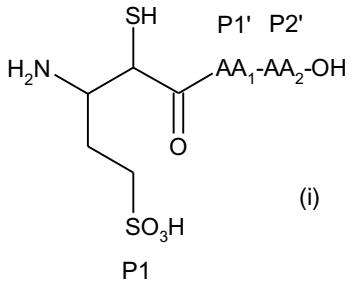
Highlights from other journals – June 2000

Subsite preferences in aminopeptidase A

Aminopeptidase A (APA) or EC 3.4.11.7, is a membrane-bound zinc metallopeptidase that specifically cleaves acidic N-terminal amino acids from peptide substrates. The enzyme has significant homology with aminopeptidase N (APN), another peptidase that cleaves hydrophobic and basic N-terminal residues from peptides. To gain a better understanding of the physiological function of APA in brain and peripheral tissues it is necessary to identify efficient and selective inhibitors. A combinatorial approach has been used to investigate the subsite preferences of APA (Investigation of subsite preferences in aminopeptidase A (EC 3.4.11.7) led to the design of the first highly potent and selective inhibitors of this enzyme, C. David *et al*., J. Med. Chem., 42, (1999), 5197-5211).

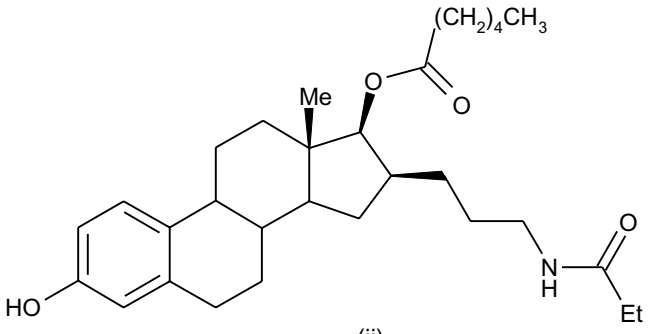


(i)

Amastatin is generally used as an APA inhibitor but this compound is not selective and in particular also has affinity for APN. This study investigated the combinatorial synthesis of thiol-containing compounds of the structure (i). It was found that the introduction of a sulphonate into the P1 position, a hydrophobic group into P1', and a (3R)-carboxyproline in P2' gave rise to very selective and efficient inhibitors for APA.

Solid-phase synthesis of phenolic steroids

Both 17β-hydroxysteroid dehydrogenase and the estrogen receptor can bind estradiol as a natural ligand/substrate and appear to play key roles in estrogen-sensitive diseases such as breast and endometrium cancers. These two targets could be blocked by two different or the same drug offering a novel therapy for these cancers. A recent paper describes a method for the solid-phase combinatorial synthesis of phenolic steroids with relevance for these protein targets (Solid-phase synthesis of phenolic steroids: from optimization studies to a convenient procedure for combinatorial synthesis of biologically relevant estradiol derivatives M.R. Tremblay and D. Poirier, *J. Comb. Chem*., 2, (2000), 48-65).

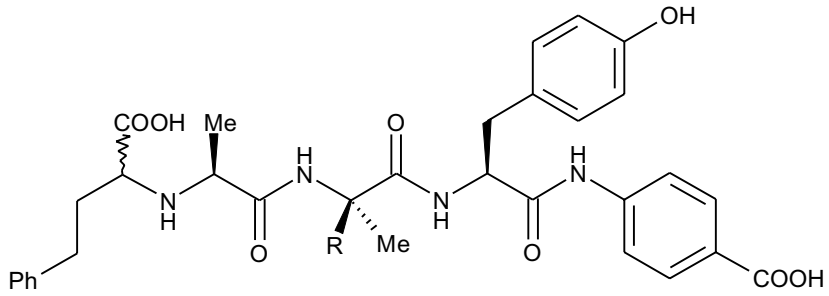


(ii)

A survey of possible solid-phase linker methods for the steroid phenol group revealed that a photolabile *o*-nitrobenzyl ether linker was most effective in generating products (e.g. ii) in excellent yields and purities. This study of library methods has set the scene for the generation of libraries of estradiol-related compounds that could be tested for affinity for inhibition of the estradiol binding proteins.

Inhibitors of EC 3.4.24.15

In vitro, the neutral metalloendopeptidase EC 3.4.24.15 hydrolyses a number of biologically active peptides including bradykinin and neurotensin. Although it may have a physiological role in brain and endocrine function, further investigations of its function have been restricted by the lack of a stable potent inhibitor. To date, the most frequently used inhibitor, N-[1-(*R,S*)-carboxy-3-phenylpropyl]-Ala-Ala-Tyr-*p*-aminobenzoate (cFP, iii), has been limited by rapid hydrolysis of the Ala-Tyr bond. Therefore, a recent study has investigated the design and solid-phase synthesis of novel stable inhibitors of EC 3.4.24.15 (Development and characterization of novel potent and stable inhibitors of endopeptidase EC 3.4.24.15, C.N. Shrimpton *et al*., *Biochem. J.,* 345, (2000), 351-356).



(iii) R = H
(iv) R = Me

cFP was used as template for the solid-phase preparation of compounds in which the scissile bond has been replaced by groups that are more stable to hydrolysis. The compound in which the Ala-Tyr amide bond had been reduced to an aminomethyl group had affinity for the enzyme reduce by some thousand-fold. However, it was found that replacement of the Ala residue with aminoisobutyric acid has given a compound (iv) with a Ki of 23 nM. Furthermore, this compound is stable to hydrolysis and does not inhibit angiotensin converting enzyme, or other related thermolysin-like or neutral endopeptidases. The compounds thus provides a valuable tool for the further investigation of the physiological function of EC 3.4.24.15.